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# When Hymenopteran Males Reinvented Diploidy

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## Summary

**In most plants and animals, a consistent relationship exists between the DNA content of a cell and its metabolic activity [1, 2]. The male-haploid sex determination of Hymenoptera and other arthropods may therefore impose a particular selective pressure upon males, which must evolve adaptations to cope with a genomic DNA reduced by half compared with that of females. Here, we show that a nuclear DNA content similar to that of females is restored in muscles of males in all hymenopteran lineages tested except the most basal one (Xyelidae). This doubling of DNA content in males does not occur in other haplodiploid insects, such as thrips (Thysanoptera) and whiteflies (Sternorrhyncha). These results indicate that this adaptation probably occurred early in hymenopteran history, possibly because males acquired strong flying and dispersal abilities.**

## Results and Discussion

In insects, an increase in DNA content by endoreduplication is observed in particularly active tissues, such as labial glands, fat bodies, Malpighian tubules, and midgut epithelium [3, 4]. Among Hymenoptera, a few early studies revealed a broad range of ploidy levels in the honeybee (*Apis mellifera*) [5–7] and in parasitic wasps [8, 9]. Interestingly, although males develop from haploid eggs, they reach endopolyploidy levels (8C, 16C, or 32C) equivalent to female levels in Malpighian tubule and intestinal cell nuclei. This indicates the importance of nuclear DNA content to individual fitness in insects. While conducting an analysis of the nuclear DNA variations in Hymenoptera, we discovered that diploidy is restored in muscles of male bumblebees (*Bombus terrestris* L.). Comparative analysis across haplodiploid insects revealed that this doubling of DNA content occurs in all tested Hymenoptera families except the most basal lineage (Xyelidae), but not in thrips (Thysanoptera) and whiteflies (Sternorrhyncha).

We used flow cytometry, a rapid and accurate method for characterization of nuclear DNA variations [10], to determine the DNA content of individual cell nuclei from various body parts of adult bumblebees (Figure 1). In females, the major peak of DNA content was equal for the head and thorax. As expected from the male-haploid sex-determining system, nuclei derived from males' heads displayed half the DNA-content of female heads and were equal to 1C-DNA. However, in males the distribution of nuclei from thorax extracts showed the major peak at 2C, as in females. Because the thorax is densely packed with flight muscles, we checked whether these and other muscles consistently possessed diploid nuclei in males. Thoracic muscles showed identical patterns for both sexes, with a major peak at 2C-DNA. This was also true for leg tissue (mostly composed of muscles) and for mandibular muscles, which must contribute to the small 2C-DNA peak observed for male heads (Table 1).

To cast light on the phylogenetic origin of this pattern, selected taxa from various hymenopteran and other haplodiploid lineages were analyzed (Figure 2). In all tested taxa, nuclei from male heads contained almost exactly half the amount of DNA of female heads (DNA index [D.I.] of approximately 0.5). By contrast, in almost all Hymenoptera the male thorax followed the bumblebee pattern, with a major peak equal to 2C-DNA, as in females (D.I. of approximately 1.0). The only exception was *Xyela curva* (Xyelidae), whose male thorax, like the male head, peaked at 1C-DNA. In two non-Hymenoptera haplodiploid taxa, thrips (Thysanoptera: Thripidae) and whiteflies (Sternorrhyncha: Aleyrodidae), nuclei contained half as much DNA in males as in females for all body parts. Because DNA endoreplication is usually negatively related to genome size [1, 11], we investigated whether substantial differences in diploid DNA content occurred among hymenopteran taxa. Although some variation is present, the 2C-DNA value of *Xyela curva* females lies within the range of the other studied Hymenoptera (Figure 2).

Our results show that diploidy is restored in muscles of males in all tested Hymenoptera lineages except the most basal one, the Xyelidae. Diploidy restoration might have allowed males to keep pace with females in terms of muscular metabolic activity and efficiency. Because insect flight requires large and metabolically very active muscles [12], males with diploid muscles would have benefited from a competitive edge in pair formation. Better predator avoidance and longer dispersal distances are other likely benefits of male flight enhancement. Consistent with this hypothesis, endoreduplication from 2C to 4C was recently reported from flight muscles of male Strepsiptera, small insects that have tiny genomes and must fly actively in search of apterous females parasitic on other insects [13]. By

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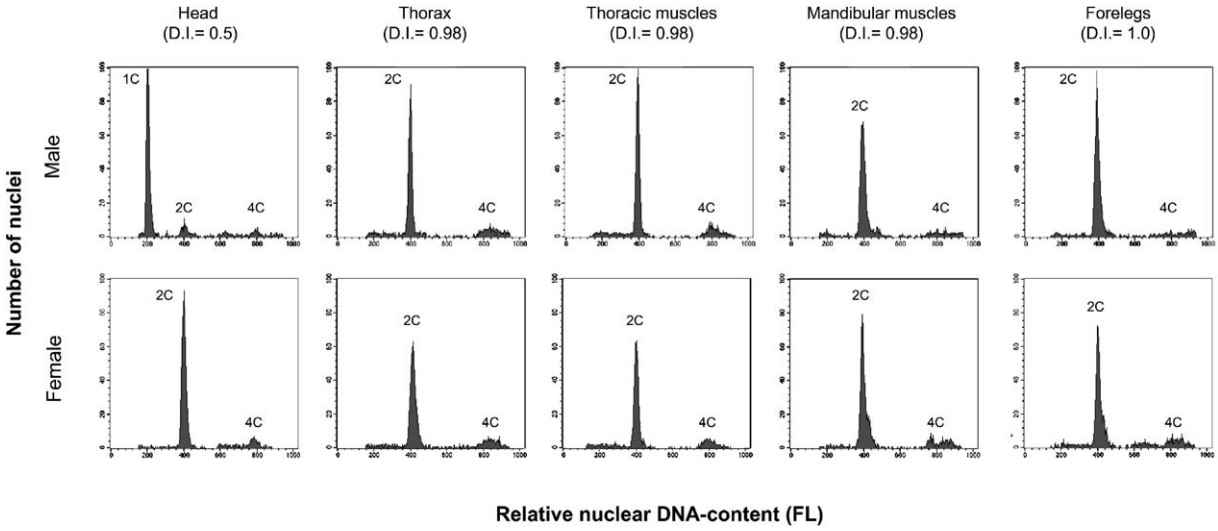



Figure 1. Flow-Cytometric DNA Histograms from Nuclear Preparations of Various Tissues of Representative Male and Female Bumblebees

The first (major) peak corresponds to the distribution of nuclei over $G_0/G_1$ (i.e., the ploidy level of the sample with 1C-DNA and 2C-DNA for males and females, respectively), and the second peak corresponds to the distribution of nuclei over $G_2$/M of the cell-cycle stages. Other peaks higher than peak 2 correspond to the distribution of polyploid nuclei. The major peak of DNA content in females was chosen as the diploid standard (2C-DNA). The flow cytometer was calibrated so that the 2C-DNA nuclei population from females yielded a relative DNA content (fluorescence intensity FL) near channel 400. See Table 1 for statistics.

contrast, in thrips and whiteflies, whose transportation on the wing depends more on wind than on directional flight [14], males remain fully haploid.

The Xyelidae, renowned for being weak flyers [15], constitute the sister group of all other living Hymenoptera [16–18]. It is therefore most parsimonious to hypothesize that the restoration of diploidy in male muscles was selected early in the stem species of all non-xyelid Hymenoptera. This hypothesis is also supported by the fact that in species whose males are unable to fly (e.g., *Nasonia vitripennis*) or in which males are moved by wind (e.g., *Trichogramma brassicae*), the males' thoracic muscles are still diploid. The possible role of endopolyploidization to diploid status in male muscles in the diversification of the Hymenoptera remains an open question.

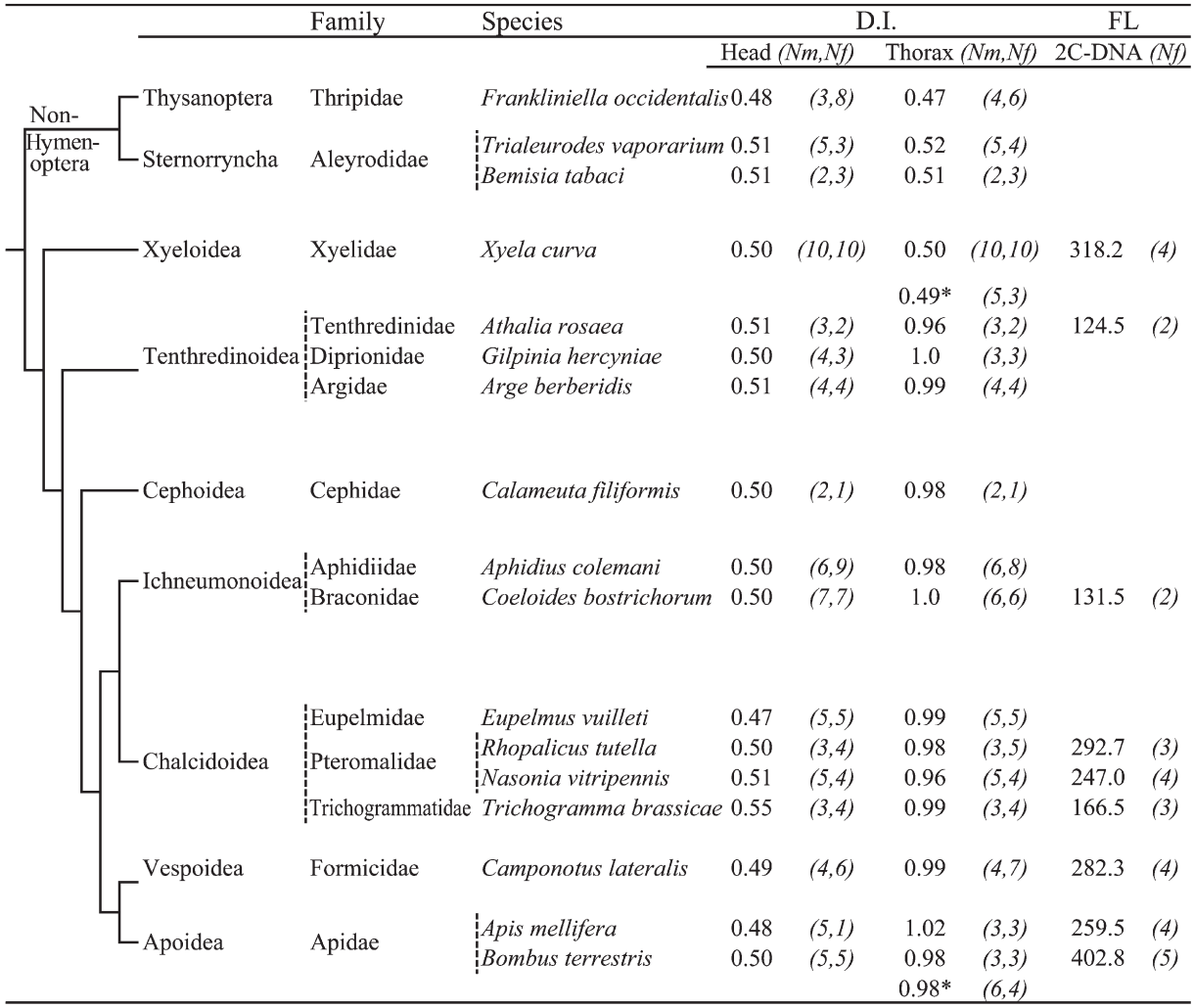
### Experimental Procedures

Tissues were obtained from either freshly killed or deep-frozen (below –20°C) individually dissected insects. The determination of DNA ploidy level was based on the measurement of nuclear DNA content of different body parts (head, thorax, and muscles) by flow

Table 1. Determination of Nuclear DNA Content by Flow Cytometry in Various Body Parts of Bumblebees

| Body part | Male | | | | | | | Female | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 1C-DNA | | 2C-DNA | | 4C-DNA | | | 2C-DNA | | 4C-DNA | |
| | | FL | Percent Nuclei | FL | Percent Nuclei | FL | Percent Nuclei | | FL | Percent Nuclei | FL | Percent Nuclei |
| Head | (5) | 201.4 ± 1.7 | 74.5 ± 4.3 | 392.2 ± 9.5 | 10.3 ± 2.2 | 785.0 ± 12.1 | 4.1 ± 0.9 | (5) | 402.8 ± 3.0 | 82.9 ± 1.7 | 795.4 ± 10.9 | 8.1 ± 1.4 |
| Thorax | (3) | — | — | 403.3 ± 4.9 | 74.5 ± 2.1 | 816.0 ± 8.9 | 16.1 ± 1.3 | (3) | 409.7 ± 5.9 | 77.5 ± 2.3 | 817.3 ± 47.0 | 9.2 ± 4.2 |
| Thoracic muscles | (6) | — | — | 396.8 ± 3.8 | 76.1 ± 6.3 | 797.6 ± 9.4 | 12.9 ± 5.0 | (4) | 403.2 ± 10.3 | 77.1 ± 2.9 | 802.7 ± 19.6 | 9.6 ± 2.5 |
| Mandibular muscles | (3) | — | — | 389.0 ± 3.4 | 82.3 ± 7.2 | 789.7 ± 47.4 | 5.7 ± 0.8 | (3) | 395.7 ± 4.5 | 77.6 ± 2.5 | 767.0 ± 6.08 | 9.8 ± 4.2 |
| Forelegs | (3) | — | — | 395.7 ± 1.5 | 82.0 ± 2.3 | 794.5 ± 16.3 | 4.6 ± 0.9 | (3) | 394.7 ± 7.0 | 77.8 ± 2.0 | 798.0 ± 41.1 | 7.9 ± 3.9 |
| Midlegs | (3) | — | — | 401.0 ± 5.3 | 80.6 ± 4.3 | 810.5 ± 5.0 | 4.6 ± 0.7 | (3) | 395.0 ± 5.6 | 80.5 ± 3.5 | 810.3 ± 40.9 | 9.3 ± 5.8 |
| Hindlegs | (3) | — | — | 402.0 ± 7.0 | 80.6 ± 4.5 | 805.5 ± 40.3 | 4.3 ± 0.9 | (3) | 409. 0 ± 2.0 | 78.2 ± 6.3 | 805.0 ± 9.5 | 9.9 ± 9.3 |

Values for relative nuclear DNA content (peak channel of fluorescence intensity FL) and percent of nuclei pertaining to each peak are given for ploidy levels of 1C- (males only; there was no such peak in females), 2C-, and 4C-DNA. Values given are means ± standard deviation. Sample size is given between parentheses (N).



| | Family | Species | D.I. Head *(Nm,Nf)* | | D.I. Thorax *(Nm,Nf)* | | FL 2C-DNA *(Nf)* | |
|---|---|---|---|---|---|---|---|---|
| Non-Hymenoptera – Thysanoptera | Thripidae | *Frankliniella occidentalis* | 0.48 | *(3,8)* | 0.47 | *(4,6)* | | |
| Non-Hymenoptera – Sternorryncha | Aleyrodidae | *Trialeurodes vaporarium* | 0.51 | *(5,3)* | 0.52 | *(5,4)* | | |
| | | *Bemisia tabaci* | 0.51 | *(2,3)* | 0.51 | *(2,3)* | | |
| Xyeloidea | Xyelidae | *Xyela curva* | 0.50 | *(10,10)* | 0.50 | *(10,10)* | 318.2 | *(4)* |
| | | | | | 0.49* | *(5,3)* | | |
| Tenthredinoidea | Tenthredinidae | *Athalia rosaea* | 0.51 | *(3,2)* | 0.96 | *(3,2)* | 124.5 | *(2)* |
| | Diprionidae | *Gilpinia hercyniae* | 0.50 | *(4,3)* | 1.0 | *(3,3)* | | |
| | Argidae | *Arge berberidis* | 0.51 | *(4,4)* | 0.99 | *(4,4)* | | |
| Cephoidea | Cephidae | *Calameuta filiformis* | 0.50 | *(2,1)* | 0.98 | *(2,1)* | | |
| Ichneumonoidea | Aphidiidae | *Aphidius colemani* | 0.50 | *(6,9)* | 0.98 | *(6,8)* | | |
| | Braconidae | *Coeloides bostrichorum* | 0.50 | *(7,7)* | 1.0 | *(6,6)* | 131.5 | *(2)* |
| Chalcidoidea | Eupelmidae | *Eupelmus vuilleti* | 0.47 | *(5,5)* | 0.99 | *(5,5)* | | |
| | Pteromalidae | *Rhopalicus tutella* | 0.50 | *(3,4)* | 0.98 | *(3,5)* | 292.7 | *(3)* |
| | | *Nasonia vitripennis* | 0.51 | *(5,4)* | 0.96 | *(5,4)* | 247.0 | *(4)* |
| | Trichogrammatidae | *Trichogramma brassicae* | 0.55 | *(3,4)* | 0.99 | *(3,4)* | 166.5 | *(3)* |
| Vespoidea | Formicidae | *Camponotus lateralis* | 0.49 | *(4,6)* | 0.99 | *(4,7)* | 282.3 | *(4)* |
| Apoidea | Apidae | *Apis mellifera* | 0.48 | *(5,1)* | 1.02 | *(3,3)* | 259.5 | *(4)* |
| | | *Bombus terrestris* | 0.50 | *(5,5)* | 0.98 | *(3,3)* | 402.8 | *(5)* |
| | | | | | 0.98* | *(6,4)* | | |

Figure 2. DNA Index and 2C-DNA Fluorescence Intensity in Haplodiploid Insects

Given are the DNA index (D.I.) corresponding to male:female ratio of the relative nuclear DNA content (peak channels) of nuclei over for $G_0/G_1$ for head, thorax, and thoracic muscles (*) in haplodiploid insects, as well as 2C-DNA fluorescence-intensity values (FL) for selected Hymenoptera by reference to the bumblebee (the 2C peak was set near channel 400 for the first-tested head of female *Bombus terrestris*). All subsequent measurements were carried out under identical instrument conditions. The number of individuals analyzed is given between parentheses. Arrangement of taxa follows recent revisions of hymenopteran phylogeny [16–18].

cytometry. Abdomens were not used because they are rich in polyploid tissues, resulting in blunt, unclear *FL* peaks. Preparation of the samples and staining conditions are described in full detail elsewhere [19]. Samples were analyzed one at a time. The DNA content of 2500 nuclei from each sample was analyzed on a Facscan (Becton Dickinson, with an air-cooled Argon ion laser emitting at 488 nm) after exclusion of nonnuclear fluorescent debris of tissues and nuclear doublets (aggregates) by pulse-shape analysis, i.e., evaluation of fluorescence area versus fluorescence width of the incoming signals.

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